REMARKS

Claims 16-31 and 35 are under examination in the present case. Claims 16-20, 22-25, 27-31, and 35 are rejected under 35 U.S.C. § 112, first paragraph. Claims 16-31 and 35 are further rejected under 35 U.S.C. § 112, second paragraph. The rejections are addressed below.

Support for the amendments

Support for the claim amendments is found throughout the specification. Support for the amendment of claims 16, 22, 27, and 30, which now recite "a rab5 nucleic acid" is found, for example, at page 6, line 6, and at page 16, lines 6-24, to page 17, lines 1-5. Support for the amendment of claims 17, which now recites "endocytic pathway" is found at page 3, lines 21-24, and at page 5, lines 8-15.

Applicants reserve the right to pursue all canceled subject matter in pending or future related applications.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 16-20, 22-25, 27-31, and 35 are rejected under 35 U.S.C. § 112, first paragraph, as lacking enablement.

Claims 16-20 and 22-25

Claims 16-20 and 22-25 are directed to methods for identifying a candidate compound useful for the treatment of Alzheimer's disease using a cell that expresses a recombinant rab5 nucleic acid. While the Office acknowledges that applicants have enabled an *in vitro* method for identifying a candidate compound as being useful for the treatment of Alzheimer's disease, the Office asserts that applicants have failed to enable a transgenic mouse expressing a recombinant rab5 nucleic acid. The Office asserts that the phenotype of a rab5 transgenic animal would be unpredictable given (i) that transgene expression is unpredictable due to position effects; (ii) that species-specific requirements for transgene design exist; and (iii) that the genetic background of the transgenic mouse

can have an unpredictable effect on the transgenic phenotype. The Office cites the following references to support its assertions regarding the unpredictability of transgene expression: Wall, *Theriogen.* 45:57-68, 1996; Sigmund, *Arterioscler. Thromb. Vasc. Biol.* June 2000: 1425-1429, 2000; Taurog et al., *J. Immunol.* 141:4020-4023, 1988; Hammer et al., *Cell* 63:1099-1112, 1990; Mullins *EMBO* 8:4065-4072, 1989; and Mullins et al., *Nature* 344:541-544, 1990. These rejections are respectfully traversed.

Positional Effects

Turning first to the Office's assertion that achieving transgene expression in the appropriate tissue, at the appropriate level, and at the appropriate time are not well established because gene transfer methods rely on genomic transgene integration; thus, transgene expression is subject to positional effects. The Office cites Wall, page 61, third paragraph, for support. A thorough reading of the entire reference, however, suggests that positional effects can easily be overcome using routine methods known to the skilled artisan.

> It is likely that both of these factors (position effect and unidentified control elements) contribute to lack of transgene expression in some lines and variable expression in other lines. Some of these problems will be obviated by use of "boundary" DNA sequences that block the influence of surrounding genes (page 62, lines 1-5).

Moreover, another reference cited by the examiner, Mullins (1989) states

> Position-dependent expression may be circumvented by the construction of a minilocus (Grosvel et al., 1987) which permits copy number-dependent expression irrespective of the insertion site (page 4070, left column, first paragraph).

Alternatively, transgenic phenotype can simply be assessed in multiple independent lines. Another reference cited by the examiner, Sigmund, teaches that a transgenic phenotype shared by multiple independent lines is unlikely to be caused by the effect of position on transgene expression.

> Consequently, it is essential that several independent lines of mice, derived from founders with different insertion sites, are examined before a conclusion relating a phenotype to a specific pattern of transgene expression is made (page 1426, left column, first paragraph).

Clearly, even references cited by the Office agree that no more than routine methods are required to overcome position-effects on transgene expression. This is insufficient basis for the present § 112, first paragraph rejections (*In re Wands*, 858 F.2d 731, 8 U.S.P.Q.2d 1400 (Fed. Cir. 1988). Accordingly, this basis for the enablement rejection should be withdrawn.

Species-specific Requirements

The second basis for the enablement rejection is the Office's assertion that unpredictable species-specific requirements for transgene design exist. The Office states that animal models of human disease have relied on transgenic rats when the development of mouse models was not feasible. The Office cites Mullins (1989) and Mullins (1990) in support of this position. Mullins (1989) teaches the effect of oestrus on DBA/2J Ren-2 gene expression in transgenic mice. Mullins (1990) also teaches that transgenic rats expressing mouse *Ren-2* are a model system for hypertension. In fact, Mullins was successful in using the same construct to generate transgenic mice and rats. Mullins (1990) makes this clear (at page 541, right column, second paragraph) stating, "We chose the mouse Ren-2 renin gene for introduction into the rat germline *because it had already been characterized in transgenic mice* and because we expected it to be highly expressed in certain tissues (emphasis added)." Mullins (1989) and (1990) fail to support a species-specific requirement for transgene design, and support applicants position that the production of transgenic animals is routine.

The Office further asserts that Mullins' (1990) investigation was preceded by the failure of Mullins' (1989) investigation, because the transgenic mice studied in 1989 failed to develop human disease-like symptoms. This assertion cannot be supported either by Mullins (1989) or Mullins (1990). Mullins (1989) states that the purpose of the study was to address the tissue-specific control of the mouse *Ren-2* gene, and teaches that this was successful since the constructs directed "significant expression within the anticipated spectrum of tissues" (page 4069, fifth paragraph), although the level of expression was below that of endogenous Ren-2. Mullins does not address whether or

not the *Ren-2*-expressing transgenic mice developed hypertension. Thus, this basis for the rejection may be withdrawn.

The Office also cites Hammer (1990) and Taurog (1988) to support its position that species-specific requirements for transgene design render a transgenic phenotype unpredictable. Hammer teaches a transgenic rat that expresses HLA-B27 and human β2-microglobulin that exhibit pathogenesis that resembles human B27-related disorders. Taurog teaches a transgenic mouse that express HLA-B27 and human β2-microglobulin, but fails to exhibit symptoms of HLA-B27-related disease. Contrary to the Office's assertion, this difference in pathogenesis between mice and rats was not due to species-specific differences in transgene expression, since both species expressed functional proteins (page 1099, right column, first and second paragraphs). At page 1099, right column, first paragraph, Hammer states that transgenic rats were developed because they were "susceptible to arthritic diseases that *cannot be elicited in mice* (Greenwald and Diamond, 1988) (emphasis added)."

Thus, it was a species-specific difference in susceptibility to arthritic diseases that was responsible for the difference in the rat and mouse transgenic phenotypes. The Office has provided no reason to doubt that the transgenic mouse of the instant invention would fail to be susceptible to the endosomal abnormalities. Moreover, applicants have shown that in fact mice *are* susceptible to endosomal abnormalities. Applicants disclose that segmental trisomy 16 mice display endosomal abnormalities typical of sporadic Alzheimer's disease (page 11, lines 8-16). For example, at page 19, line 20, to page 20, line 5, applicants show that at two months of age, mice with segmental trisomy 16 have enlarged early endosomes similar to those seen in sporadic Alzheimer's disease, and that at six months of age these mice have endosomal alterations within the majority of neurons of the neocortex and basal forebrain. Additionally, the mice have abnormalities in proteins associated with the regulation of the endocytic pathway (page 20, lines 21-23).

With respect to the effects of rab5 on the endocytic pathway, applicants provide working examples showing that overexpression of rab5 in murine L cells produces

increased endocytosis that resembles that observed in sporadic Alzheimer's disease (page 16, lines 6-17). The Office has provided no reason to doubt that expression of a recombinant rab5 transgene in a transgenic mouse would fail to elicit increased endocytic pathway activity that resembles changes observed in murine cells in culture. Thus this basis for the enablement rejection should also be withdrawn.

Genetic Background

The final basis for the enablement rejection is the Office's assertion that the genetic background of the transgenic mouse can have an unpredictable effect on the expressed phenotype. The Office cites Sigmund for support. A thorough reading of Sigmund suggests that while genetic background influences the phenotype of a transgenic mouse, this influence does not necessarily make the transgenic mouse's phenotype unpredictable. For example, Sigmund cites an example of the way in which genetic background influences the phenotype of a mouse having a p53 deletion (page 1425, left column, second paragraph).

> Studies performed over the past few years have clearly illustrated that phenotypes caused by specific genetic modifications are strongly influenced by genes unlinked to the target locus. For example, whereas deletion of the p53 tumor suppressor gene causes *a dramatic increase in the frequency of tumor formation* in those mice compared with wild-type mice, the types of tumors formed, their numbers per animal and age of tumor onset vary in different genetic backgrounds. (Emphasis added.)

The presence of some variability in phenotype-expression in the various genetic backgrounds *did not* make the phenotype of a mouse having a p53 deletion unpredictable. In all cases, the deletion caused "a dramatic increase in the frequency of tumor formation." Thus, the Office's conclusion, that minor variability in the expression of the phenotype renders the phenotype unpredictable, is unfounded. Moreover, the problems in genetic variability that Sigmund refers to arise, primarily, (page 1425, right column, first paragraph) "when interpreting and comparing the results of transgenic and knockout studies *between laboratories*."

Sigmund further teaches that routine methods exist to overcome such problems, and minimize variability (page 1426, right column, last paragraph, to 1428, right column first paragraph). For example, Sigmund teaches, at page 1426, right column, second paragraph, to page 1427, left column, first and second paragraphs, that experimental mice should be compared to genetically identical control mice using congenic strains. Because methods for generating genetically similar control mice are known to the skilled artisan, and since minor variations in phenotype expression fail to support the Office's assertion that the phenotype of the rab5 transgenic mouse is unpredictable, this basis for the enablement rejection should also be withdrawn.

In sum, none of the references cited supports the Office's assertion that the phenotype of a rab5 transgenic mouse would be unpredictable. Applicants refer the Office to the M.P.E.P. 2164.04, in which it is stated that the Office has the initial burden to establish a reasonable basis to question the enablement provided for the claimed invention. This is highlighted by a quotation from *In re Marzocchi* 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971), in which the courts stated:

> [I]t is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain why it doubts the truth of accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement.

This burden has not been met by the Office and the enablement rejection should be withdrawn.

Methods for Producing a rab5 Transgenic Mouse

Applicants' specification provides an enabling description that would instruct the skilled artisan how to make a transgenic mouse expressing a recombinant rab5 transgene (pages 17-19). Applicants also disclose a working example, an exemplary transgenic mouse that expresses the 46 kDa mannose 6-phosphate (MPR46) receptor under the control of the Thy-1.1 promoter (page 18, line 11, to page 19, line 4). This exemplary transgenic mouse has a phenotype consistent with an endosomal abnormality. Expression

of recombinant MPR46 in the neurons of this mouse resulted in an increase in brain levels of amyloid β peptide. It is likely that this increased MPR46 expression resulted in the redistribution of lysosomal hydrolase, which then caused an increase in Aβ production. Applicants also provide methods for assessing endocytic pathway function.

In addition, applicants provide methods for evaluating endocytic function. For example, at page 16, lines 6-10, applicants teach that immunolabeling for rab5 can be used to evaluate endosome size, and that enlarged endosomes are associated with endocytic changes associated with sporadic Alzheimer's disease. At page 16, lines 15-17, applicants teach that FITC-dextran and transferrin can be used to measure uptake of fluid phase markers and receptor mediated endocytosis.

Moreover, applicants teach methods for selecting compounds that alter the activity of the endocytic pathway in a rab5 transgenic mouse. At page 16, line 18, to page 17, lines 1-5, applicants teach that compounds that counteract the effect of rab5 overexpression on endocytosis are considered useful in the methods of the invention.

Applicants also provide a working example showing that mice are susceptible to endocytic pathogenesis. Applicants teach that segmental trisomy 16 mice display endosomal alterations characteristic of sporadic Alzheimer's disease (page 11, lines 8-16, and at page 19, line 20, to page 20, line 5). Given that mice are susceptible to endosomal alterations, and given applicants' finding that *in vitro* overexpression of rab5 in murine L cells produces changes in the endocytocytic pathway that resemble those observed in sporadic Alzheimer's disease (page 16, lines 6-17), the skilled artisan could reasonably predict that overexpression of rab5 in a transgenic mouse would produce endocytic pathway alterations.

Phenotype of rab5 Overexpressing Mouse

As evidenced in the Declaration of Dr. Ralph Nixon, under 37 C.F.R. § 1.132, applicants have successfully reduced to practice the overexpression of rab5 in mice. Specifically, applicants have demonstrated that mice overexpressing rab5 exhibit endosomal changes that resemble changes observed in Alzheimer's disease. The successful acute overexpression of rab5 in mouse brains was achieved using Herpes

Simplex Virus (HSV) infection *in vivo*. This overexpression persisted for at least ten days and involved cell populations at a considerable distance from the injection site. When highly overexpressed, rab5 was largely cytoplasmic. At lower expression levels rab5 was present in endosomes, which were substantially enlarged. Given these results, one skilled in the art would predict that rab5 transgene expression, driven by appropriate promoters, would also enlarge endosomes in rab5 overexpressing transgenic mice.

The results of these studies are shown in Exhibit A, panels A-E. Panels A and B are photomicrographs showing rab5 immunostaining in striatal neurons of sectioned mouse brains. Panel A shows low levels of rab5 present in striatal neurons in wild-type mouse brain. Panel B shows high levels of rab5 immunostaining in mouse brain sectioned three days after an HSV vector driving rab5 (HSV-rab5) expression was injected into the cingulate cortex. Panel C is a photomicrograph showing rab5 immunostaining in enlarged endosomes visualized using Nomarski optics in an HSV-rab5 injected mouse. The enlarged endosomes present in rab5 overexpressing mice resemble endosomal changes observed in Alzheimer's disease (as shown in Panel D). Panels D and E are photomicrographs showing rab5 immunostaining in human pyramidal neurons. Panel D shows rab5 immunostaining in endosomes of a normal brain. Panel E shows rab5 immunostaining in endosomes of a human brain with Alzheimer's disease.

Given applicants' results expressing rab5 *in vitro* and *in vivo*, there is no reason to doubt that a rab5 transgenic mouse would display endosomal abnormalities. Thus, this basis for the enablement rejection should be withdrawn.

Compound Selection

Applicants teach methods for using rab5 transgenic mice in selecting compounds useful for the treatment of sporadic Alzheimer's disease. Compounds are selected for their ability to counteract the effect of rab5 overexpression on endocytosis (page 16, lines18-24, and page 17, lines 1-5). Applicants also provide methods for evaluating endocytosis in a transgenic mouse (page 33, lines 11-23). In particular, endocytosis can be evaluated by determining whether endosomes are enlarged (page 16, lines 6-10, and at page 32, lines 3-6); by measuring the uptake of fluid phase markers, or by measuring

receptor-mediated endocytosis (page 16, lines 15-17, and at page 32, lines 7-19). In addition, applicants teach that compounds that counteract rab5's effect on the endocytic pathway are likely to be useful for the treatment of sporadic Alzheimer's disease.

In sum, provided with applicants' specification, the skilled artisan could predictably generate a rab5 transgenic mouse, and use it to select for compounds useful for the treatment of Alzheimer's disease. Accordingly, this rejection should be withdrawn.

Claims 27-31 and 35

Claims 27-31 and 35 are directed to methods for identifying a candidate compound as a compound that is useful for the treatment of Alzheimer's disease using a transgenic mouse that expresses a recombinant rab5 nucleic acid.

The Office asserts that applicants have failed to enable methods for using a rab5 transgenic mouse in compound screening. This rejection is respectfully traversed.

As detailed above, references cited by the Office failed to support the Office's assertion that the phenotype of a rab5 transgenic mouse would be unpredictable. Provided with the teaching of applicants' specification and using nothing more than standard techniques, one skilled in the art would be able to make and use the claimed invention to its fullest extent. As described in detail above, applicants provide methods for producing a rab5 transgenic mouse that expresses the rab-5 transgene in neurons under the control of, for example, the Thy-1.1 promoter; applicants provide methods for evaluating endocytic pathway activity in a transgenic mouse; applicants teach methods for selecting compounds that alter the activity of the endocytic pathway in a rab5 transgenic mouse; and applicants teach that selected compounds that counteract the effect of rab overexpression on endocytosis are considered useful in the methods of the invention. Moreover, applicants disclose that mice are susceptible to endocytic pathway pathogenesis and that expression of a rab5 transgene in murine cells *in vitro* produces endosomal alterations. Given this disclosure, it is reasonable to predict that a transgenic mouse expressing a recombinant rab5 transgene would display endocytic pathway

alterations. Accordingly, this rejection should be withdrawn.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 16-31 and 35 are further rejected under 35 U.S.C. § 112, second paragraph, as being indefinite.

The rejection of independent claims 16, 22, 27, 30, and 35, and their dependent claims 17, 19-22, 24-26, 28, and 32-34 has been overcome by the amendment of claims 16, 22, 27, 30, and 35.

Claims 16-31 and 35 are presently directed solely to rab5.

Claims 16-31, and 35 now recite "a compound that may be useful."

Claim 17 has been amended to correct antecedent basis.

Claims 18, 23, 29, and 31 have been cancelled.

Accordingly, the indefiniteness rejections may be withdrawn.

CONCLUSION

Applicants submit that this case is in condition for allowance, and such action is respectfully requested.

A marked-up version indicating the amendments to the claims is attached. A clean version of all pending claims is also attached.

Enclosed is a petition to extend the period for replying for three months, to and including January 8, 2003.

If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: Jan. 8, 2003

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16. (Amended) A method for identifying a candidate compound as a compound that is useful for the treatment of Alzheimer's disease, said method comprising the steps of:

(a) providing a cell expressing a recombinant rab5 nucleic acid that increases activity of the endocytic pathway;

(b) contacting said cell with a candidate compound; and

(c) measuring said activity, wherein a decrease in said activity, relative to the activity of the endocytic pathway in a cell expressing the recombinant nucleic acid but not contacted with the candidate compound, identifies the candidate compound as a compound that is useful for the treatment of Alzheimer's disease.

17. (Amended) The method of claim 16, wherein said activity of the [endosomal] endocytic pathway is selected from the group consisting of endosomal fusion, endosomal recycling, expression of MPR46, accumulation of lysosomal hydrolases in early endosomes, and accumulation of Aβ in early endosomes.

22. (Amended) A method for identifying a candidate compound as a compound that is useful for the treatment of Alzheimer's disease, said method comprising the steps of:

(a) providing a cell expressing a recombinant rab5 nucleic acid that increases activity of the endocytic pathway;

(b) contacting said cell with a candidate compound; and

(c) measuring Aβ formation, wherein a decrease in Aβ formation, relative to Aβ formation by a cell expressing the recombinant nucleic acid but not contacted with the candidate compound, identifies the candidate compound as a compound that is useful for the treatment of Alzheimer's disease.

27. (Amended) A method for identifying a candidate compound as a compound that is useful for the treatment of Alzheimer's disease, said method comprising the steps of:

(a) providing a mouse expressing a transgene comprising a recombinant rab5 nucleic acid that increases activity of the endocytic pathway;

(b) administering a candidate compound to said mouse; and

(c) measuring said activity, wherein a decrease in said activity, relative to activity in a mouse expressing said transgene but not contacted with said candidate compound, identifies the candidate compound as a compound that is useful for the treatment of Alzheimer's disease.

30. (Amended) A method for identifying a candidate compound as a compound that is useful for the treatment of Alzheimer's disease, said method comprising the steps of:

(a) providing a mouse expressing a transgene comprising a recombinant rab5 nucleic acid that increases activity of the endocytic pathway;

(b) administering a candidate compound to said mouse; and

(c) measuring Aβ formation, wherein a decrease in said Aβ formation, relative to Aβ formation in a mouse expressing said transgene but not contacted with said candidate compound, identifies the candidate compound as a compound that is useful for the treatment of Alzheimer's disease.

35. (Amended) The method of claim 34, wherein said [animal] mouse is a Tn65Dn mouse [or a mouse expressing a transgene comprising a recombinant nucleic acid] that increases activity of the endocytic pathway.

16. A method for identifying a candidate compound as a compound that is useful for the treatment of Alzheimer's disease, said method comprising the steps of:

(a) providing a cell expressing a recombinant rab5 nucleic acid that increases activity of the endocytic pathway;

(b) contacting said cell with a candidate compound; and

(c) measuring said activity, wherein a decrease in said activity, relative to the activity of the endocytic pathway in a cell expressing the recombinant nucleic acid but not contacted with the candidate compound, identifies the candidate compound as a compound that is useful for the treatment of Alzheimer's disease.

17. The method of claim 16, wherein said activity of the endocytic pathway is selected from the group consisting of endosomal fusion, endosomal recycling, expression of MPR46, accumulation of lysosomal hydrolases in early endosomes, and accumulation of Aβ in early endosomes.

19. The method of claim 16, wherein said cell is from a cell line selected from the group consisting of a fibroblast cell line, a neuronal cell line, and a neuroblastoma cell line.

20. The method of claim 16, wherein said cell is selected from the group consisting of a fibroblast, a neuron, and an endothelial cell.

21. The method of claim 16, wherein said cell is *in vitro*.

22. A method for identifying a candidate compound as a compound that is useful for the treatment of Alzheimer's disease, said method comprising the steps of:

(a) providing a cell expressing a recombinant rab5 nucleic acid that increases activity of the endocytic pathway;

(b) contacting said cell with a candidate compound; and

(c) measuring Aβ formation, wherein a decrease in Aβ formation, relative to Aβ formation by a cell expressing the recombinant nucleic acid but not contacted with the candidate compound, identifies the candidate compound as a compound that is useful for the treatment of Alzheimer's disease.

24. The method of claim 22, wherein said cell is from a cell line selected from the group consisting of a fibroblast cell line, a neuronal cell line, and a neuroblastoma cell line.

25. The method of claim 22, wherein said cell is selected from the group consisting of a fibroblast, a neuron, and an endothelial cell.

26. The method of claim 22, wherein said cell is *in vitro*.

27. A method for identifying a candidate compound as a compound that is useful for the treatment of Alzheimer's disease, said method comprising the steps of:

(a) providing a mouse expressing a transgene comprising a recombinant rab5 nucleic acid that increases activity of the endocytic pathway;

(b) administering a candidate compound to said mouse; and

(c) measuring said activity, wherein a decrease in said activity, relative to activity in a mouse expressing said transgene but not contacted with said candidate compound, identifies the candidate compound as a compound that is useful for the treatment of Alzheimer's disease.

28. The method of claim 27, wherein said activity of the endocytic pathway is selected from the group consisting of endosomal fusion, endosomal recycling, expression

of MPR46, accumulation of lysosomal hydrolases in early endosomes, and accumulation of Aβ in early endosomes.

30. A method for identifying a candidate compound as a compound that is useful for the treatment of Alzheimer's disease, said method comprising the steps of:

(a) providing a mouse expressing a transgene comprising a recombinant rab5 nucleic acid that increases activity of the endocytic pathway;

(b) administering a candidate compound to said mouse; and

(c) measuring Aβ formation, wherein a decrease in said Aβ formation, relative to Aβ formation in a mouse expressing said transgene but not contacted with said candidate compound, identifies the candidate compound as a compound that is useful for the treatment of Alzheimer's disease.

35. The method of claim 34, wherein said mouse is a Tn65Dn mouse that increases activity of the endocytic pathway.

EXHIBIT A